UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/555,574	10/23/00	BEHR J	0652.2090000

HM22/0705
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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1635	9

DATE MAILED: 07/05/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

RE Started time

DETAILED ACTION

Election/Restrictions

1. Restriction to one of the following inventions is required:

   I. Claims 1-33 and 40-46, drawn to a particle for transfection higher eukaryotic cells, classified in class 935, subclass 54, for example.

   II. Claims 34-39 and 47, drawn to a transfection particle carrying one or more endosomolytic function, classified in class 935, subclass 54, for example.

These inventions are distinct, each from the other because of the following reasons:

Inventions I and II are related as combination and subcombination, respectively. Inventions in this relationship are distinct if it can be shown that (1) the combination as claimed does not require the particulars of the subcombination as claimed for patentability, and (2) that the subcombination has utility by itself or in other combinations. In the instant case, the combination as claimed does not require the particulars of the subcombination as claimed because the ability of the transfection particle, as claimed in Group I, to enter a cell does not require endosomal lytic function. The process of endosomal lysis does not pertain to the process or characteristic of a transfection particle to enter a cell. The subcombination, Group II, has separate utility such as a designated endosomal lysis agent, for example. Polycationic molecules can be used to study endosome formation and/or used to package materials for lysosomal degradation, for example.

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art, restriction for examination purposes as indicated is proper.

During a telephone conversation with Al Schwatz on June 27, 2001 a provisional election was made with traverse to prosecute the invention of Group I, claims 1-33 and 40-46. Affirmation of this election must be made by applicant in replying to this Office action. Claims 34-39 and 47 are withdrawn from further consideration by the examiner as being drawn to a non-elected invention.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> *A person shall be entitled to a patent unless –*
>
> *(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.*

2. Claims 1-6, 19, 22-29, 40, 41, 45 and 46 are rejected under 35 U.S.C. 102(b) as being anticipated by Gershon *et al.* (Biochemistry, Vol 32, p. 7143-7151, 1993).

Claims 1-6 are drawn to a particle for transfecting higher eucaryotic cells with nucleic acids such as DNA, plasmid DNA, or RNA *in vitro* and *in vivo* comprising organic cationic molecules (claim 1, claim 24 for DNA, claim 25 for plasmid DNA, claim 26 for RNA) wherein the cationic molecules are lipids obtained by dimerization or oligomerization of cationic detergent molecules (claim 2) connected by a linkage that is degradable under cellular conditions (claim 22) wherein the cationic detergent precursor molecules comprise a) at least one function for binding, b) at least one lipophilic residue, c) a non-toxic recipient backbone, d) a cationic group for binding nucleic acids (claim 3). The claimed function of the cationic molecule is selected from thiols, acid hydrazides, aldehydes, amines, and ethylene residues (claim 4), or from an amine or derivative thereof (claim 6), or is a polyamine (claim 19) and the lipophilic residue is selected from lipophilic amides, ester, or ethers (claim 5). The claimed transfection particle carries one or more cellular targeting functions and/or one or more functions capable of facilitating endocytosis (claim 27) and is linked to cationic molecules that are linked to nucleic acid binding (claims 28 and 29). Claims 45 and 46 are drawn to a kit comprising one or more nucleic acid molecules, one or more cationic precursor molecules, suitable buffers, and other reagents useful for preparation, purification, and *in vitro* or *in vivo* application of a transfection particle of claim 1 (claim 45) or further comprising more functions for cellular targeting (claim 46).

Gershon *et al.* discloses cationic liposomes for the transfection of DNA and RNA into a large variety of eukaryotic cells wherein said liposomes enter the cell through liposome vesicle mediated endocytosis (see abstract). The negatively-charged nucleic acids are thermodynamically driven to liposomal encapsulation through electrostatic interactions with

cationic liposomal precursor molecules (p. 7143, paragraph 1). Nucleic acids used in Gershon *et al.* include the plasmid *pBlueScript* (p. 7144, paragraph 2). The liposomes used in Gershon *et al.* were prepared from either *N*-[1-[2,3-Bis(oleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride, DOTMA, or from phosphatidylethanolamine, PE (p. 7144, paragraph 3), which have detergent properties and thus, are considered detergent molecules. Liposome formation was facilitated by oligomerization of the DOTMA and PE precursor molecules and DNA, RNA, and plasmid DNA encapsulation into said liposomes was achieved without crosslinking (p. 7143, paragraph 1 and p. 7144, paragraph 2). Both DOTMA and PE comprise of at least one functionality for binding to the same or different precursor molecules, at least one lipophilic residue (PE contains a C_{15} ester chain and DOTMA contains a C_{17} ether chain), a biologically compatible backbone, and a cationic group. Furthermore, both DOTMA and PE contain ethylene and amine functionalities. As stated above, the C_{15} chain of PE belongs to an ester group and the C_{17} chain of DOTMA belongs to an ether group. The cationic group of both PE and DOTMA is a protonated amine (NH_3^+). Moreover, Gershon *et al.* discloses said cationic liposomes and said cellular targeting cationic liposomes in Tris buffer at pH 7.5.

Therefore, the invention of the above claims is anticipated by Gershon *et al.*

3. Claim 7 is rejected under 35 U.S.C. 102(b) as being anticipated by DePrince *et al.* (U.S. Patent No. 7,705,693).

Claim 7 is drawn to said transfection particle wherein the function for binding to nucleic acid molecule is guanidine.

DePrince *et al.* disclose cationic lipids for transfer of nucleic acids into cells. The cationic lipids disclosed in DePrince *et al.* contain a guanido moiety (column 1, line 50, structure F).

Therefore, the invention of the above claim is anticipated by DePrince *et al.*

4. Claim 20 is rejected under 35 U.S.C. 102(b) as being anticipated by Yoshikawa *et al.* (FEBS Letters, Vol. 396, p. 71-76, 1996).

Claim 20 is drawn to said transfection particle wherein the cationic precursor is a spermine derivative.

Yoshikawa *et al.* discloses a transfection particle containing the spermine derivative, dioctadecylamidoglycylspermine (see abstract).

Therefore, the invention of the above claim is anticipated by Yoshikawa *et al.*

5. Claims 30-33 is rejected under 35 U.S.C. 102(b) as being anticipated by Hawley-Nelson *et al.* (U.S. Patent No. 5,736,392 and U.S. Patent No. 6,051,429 which is a CIP of U.S. Patent No. 5,736,392).

Claims 30-33 are drawn to said transfection particle characterized in that it carries one or more cellular targeting function that is a cellular protein ligand (claim 30), sugar residue (claim 31), galactose (claim 32), or mannose (claim 33).

Hawley-Nelson *et al.* discloses cationic transfection particles for delivery of nucleic acids to intracellular and extracellular targets (see abstract). Additionally, Hawley-Nelson *et al.* discloses examples of receptor-ligand targets in Table 1 (column 7, line 25) which consist of

fibronection and homologues, vitronectin, laminin 3, tenascin 1, collagen 1, collagen 6, von Wildebrand factor, fibrinogen 1, and thrombospondin. The ligands recited above are glycoproteins which comprise of complex oligosacchrides composed of monomeric sugar molecules, such as glycosminoglycan in the case of fibrinogen.

Therefore, the invention of the above claims are anticipated by Hawley-Nelson *et al.*

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> *The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.*

6. Claims 8-18 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for guanidyl-cysteine-decylamide ($C_{10}C^{G+}$) and ornithyl-cysteine-dodecylamide ($C_{12}CO$), does not reasonably provide enablement for the claimed molecules with recited functionalities claimed in the instant invention represented by general formula I recited in claim 8. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the application coupled with information known in the art without undue experimentation (See *United States v. Telectronics Inc.,* 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based upon a single factor, but rather is a conclusion reached by weighing many factors. The Courts, in *Ex parte Forman,* 230

USPQ 546 (Bd. Pat. App. & Inter. 1986) and later *In re Wands,* 8 USPQ2d 1400 (Fed. Cir. 1988) described the standard of undue experimentation as a standard of reasonableness and set forth the various factors to be considered in the determination of enablement for an invention. These factors include the following:

1) State of the prior art. The prior art in the general area of lipophilic cationic transfection particles is well understood for particular lipophilic cationic molecules, as evidenced by the prior art citations recited above in this Office Action, for example.

2) Unpredictability of the art. The art of transfection using lipophilic cationic molecules is unpredictable. Successful achievement of transfection is highly dependent upon the chemical, biophysical, biochemical, and biological properties of the lipophilic cationic molecule of interest. These properties discussed in a the review reference by Zelphati *et al.* (Journal of Liposome Research, Vol. 7, p. 31-49, 1997) include, but are not limited to, the ability of precursor cationic molecules to oligomerize based upon the extent of hydrophobic interactions, compaction and encapsulation with nucleic acids, cellular uptake and internalization, for instance. As outlined in the review reference by Zelphati *et al.* and in the references cited above in the Office Action, for example, only a small number of lipophilic cationic molecules have been demonstrated to be effective for the use of transfection in higher eukaryotic cells. Zelphati *et al.* expressly recites a limited number of compounds such as dimethyldioctaecylammonium bromide (DDAB), dioleylphosphatidylethanolamine (DOPE), and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) (refer to p.33, paragraph 1), for instance.

While applicants have demonstrated the capability of guanidyl-cysteine-decylamide ($C_{10}C^{G+}$) and ornithyl-cysteine-dodecylamide ($C_{12}CO$) to be made and used of eukaryotic cells *in*

vitro (Example 1 on p. 45, Example 9 on p. 69, Example 10 on p. 10, Example 13 on p. 71, Example 14 on p. 72, Example 15 on p. 73, and Example 20 on p. 79), applicants have not provided guidance in the specification or examples that would show by correlation the practice of the instant invention the capability of **all** the cationic molecules commensurate in scope with claims 8-18. In particular, applicants have not addressed the unpredictable factors recited above such as the ability of precursor cationic molecules to oligomerize based upon the extent of hydrophobic interactions, compaction and encapsulation with nucleic acids, cellular uptake and internalization, for instance.

3) Breadth of the claims. The broadest claim reads on a transfection particle wherein the cationic precursor is represented by general formula I recited in claim 8. Substituients R_1-R_3, X, Y, Z, and further R_4-R_6, and combinations thereof are recited in claims 9-11.

4) Number of working examples. Applicants have provided working examples only for guanidyl-cysteine-decylamide ($C_{10}C^{G+}$) and ornithyl-cysteine-dodecylamide ($C_{12}CO$) to be made and used of eukaryotic cells *in vitro* (Example 1 on p. 45, Example 9 on p. 69, Example 10 on p. 10, Example 13 on p. 71, Example 14 on p. 72, Example 15 on p. 73, and Example 20 on p. 79). Applicants have not provided examples for **all** the cationic molecules represented by general formula I recited in claim 8 with substituients R_1-R_3, X, Y, Z, and further R_4-R_6, and combinations thereof recited in claims 9-11.

5) Amount of guidance presented by applicants. Applicants present no guidance on how the skilled artisan would overcome problems recognized in the art as outlined above such as the ability of precursor cationic molecules to oligomerize based upon the extent of hydrophobic

interactions, compaction and encapsulation with nucleic acids, cellular uptake and internalization, for instance.

6) Level of skill in the art. The level of skill in the art is high. It must be considered that the skilled artisan, in order to practice the claimed invention, would have had to practiced undue trial and error experimentation with little or no guidance from the prior art.

7) Nature of the invention. Thenature of the invention involves some of the most complex aspects of molecular biology, organic chemistry, biophysics, and biochemistry, i.e. chemical synthesis of precursor cationic molecules, spectrochemical analysis of DNA/cationic molecules, transfection.

Given the above analysis of the factors which the Courts have determined are essential for determining whether a specification provides enablement for a claimed invention, it must be considered that the skilled artisan would have had to have practiced trial and error experimentation, with little or no guidance from the prior art, in order to try to practice the claimed invention. Such experimentation is the antithesis of enablement under 35 USC 112, first paragraph, and said experimentation must be considered to be undue and excessive.

7. Claims 42-44 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 42 and 43 are drawn to a pharmaceutical composition comprising said transfection particle complexed to a plasmid encoding a therapeutically active protein. Claim 44

is drawn to a method for introducing therapeutically active nucleic acid into a mammal wherein said transfection particle is administered to said mammal intradermally.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the application coupled with information known in the art without undue experimentation (See *United States v. Telectronics Inc.,* 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based upon a single factor, but rather is a conclusion reached by weighing many factors. The Courts, in *Ex parte Forman,* 230 USPQ 546 (Bd. Pat. App. & Inter. 1986) and later *In re Wands,* 8 USPQ2d 1400 (Fed. Cir. 1988) described the standard of undue experimentation as a standard of reasonableness and set forth the various factors to be considered in the determination of enablement for an invention. These factors include the following:

1) State of the prior art. The prior art in the area of pharmaceutical use of cationic liposomes for nucleic acid delivery *in vivo* as a therapeutic agent is totally undeveloped. As stated in the first sentence of the abstract disclosed in the review reference by Zelphati *et al.* (Journal of Liposome Research, Vol. 7, p. 31-49, 1997), "cationic liposomes are a useful *in vitro* but as yet unproven *in vivo* delivery system for oligonucleotides.

2) Unpredictability of the art. The art art in the area of pharmaceutical use of cationic liposomes for nucleic acid delivery *in vivo* as a therapeutic agent is extremely unpredictable. Enablement of applicants' invention required that the claimed transfection particles be able to target particular diseased cells, enter said cells without significant cellular toxicity or degradation to the encapsulated nucleic acids, and facilitate specific cellular activity to mediate a therapeutic effect, as discussed in Zelphati *et al.,* for example. While applicants have demonstrated *in vitro*

delivery of DNA to a number of eukaryotic cells, such as SKOV3, BNLCL.2, and K562 cells for instance, with guanidyl-cysteine-decylamide ($C_{10}C^{G+}$) and ornithyl-cysteine-dodecylamide ($C_{12}CO$) (Example 1 on p. 45, Example 9 on p. 69, Example 10 on p. 10, Example 13 on p. 71, Example 14 on p. 72, Example 15 on p. 73, and Example 20 on p. 79), applicants have not provided guidance in the specification or examples that would show by correlation the pharmaceutical usage or methods to achieve therapeutic effects with any of the claimed transfection particles.

3) Breadth of the claims. The broades claim reads on a pharmaceutical composition comprising said transfection particle, a method for introducing therapeutically active nucleic acid into a mammal wherein said transfection particle is administered to said mammal intradermally, and a kit useful for preparation, purification and *in vitro* or *in vivo* application of said transfection particle comprising functions for cellular targeting or endosomolytic functions.

4) Number of working examples. Applicants provide no working examples of the claimed invention. It is noted that Example 19 describes the intradermal delivery of C10CG+ in mice; however, there is no description or guidance within the example to demonstrate a therapeutic effect used in the prevention, diagnosis, alleviation, treatment or cure of disease.

5) Amount of guidance presented by applicants. Applicants provide no working examples of the claimed invention. Again, it is noted that Example 19 describes the intradermal delivery of C10CG+ in mice; however, there is no description or guidance within the example to demonstrate a therapeutic effect used in the prevention, diagnosis, alleviation, treatment or cure of disease.

6) Level of skill in the art. The level of skill in this art is extremely high. It must be considered that the skilled artisan, in order to practice the claimed invention, would have had to have practiced undue trial and error experimentation with little or no guidance from the prior art.

7) Nature of the invention. The nature of the invention involves some of the most complex aspects of medicine, molecular biology, organic chemistry, biophysics, and biochemistry, i.e. gene therapy, chemical synthesis of precursor cationic molecules, spectrochemical analysis of DNA/cationic molecules, transfection.

Given the above analysis of the factors which the Courts have determined are essential for determining whether a specification provides enablement for a claimed invention, it must be considered that the skilled artisan would have had to have practiced trial and error experimentation, with little or no guidance from the prior art, in order to try to practice the claimed invention. Such experimentation is the antithesis of enablement under 35 USC 112, first paragraph, and said experimentation must be considered to be undue and excessive.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> *The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.*

8. Claims 3, 4, 6, 7, 28, 29, 30, 31, 46 and 47 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. It is unclear if the term "function" refers to an action facilitated by the transfection particle or if the term "function" refers to a property, i.e. chemical functionality, of the transfection particle.

Additionally, claim 3 recites "...a non-toxic recipient backbone." The term "non-toxic" is indefinite and vague because it is unclear to what and/or whom toxicity refers to or at what concentrations and/or dosages meet the non-toxicity requirement.

Furthermore, claim 4 recites "... residues that are suitably substituted to provide enamines." The term "suitably substituted" is indefinite and vague because it is unclear what the term "suitable" entails.

9. Claims 8-10, 13, and 14 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The above claim recite "radical" and it is unclear that radical formation was described in the specification, in particular the synthesis of said cationic transfection particles.

In addition, claims 9 and 11 recite "...molecules correspond to general formula I." The term "suitably substituted" is indefinite and vague because the extent to which the cationic precursor molecules corresponding to formula I is unclear. Recitation of "suitably substituted" is indefinite because it fails to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

10. Claims 22, 28, and 29 recite the limitation "linkages" or "linked" in reference to a cationic molecule or function, respectively. There is insufficient antecedent basis for this limitation in the claim.

11. Claim 27 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. It is unclear if the term "carries" refers to an action facilitated by the transfection particle or if the term "carries" refers to a property, i.e. biological activity, of the transfection particle.

Objections

12. Claim 21 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitation of the base claim and any intervening claims.

No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lauren Nguyen, Ph.D. whose telephone number is 703-308-0256. The examiner can normally be reached on Monday-Friday 9-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John LeGuyader can be reached on 703-308-0447. The fax phone numbers for the organization where this application or proceeding is assigned are 703-308-4242 for regular communications and 703-305-7939 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0196.

Lauren Nguyen, Ph.D.
July 2, 2001

DAVID GUZO
PRIMARY EXAMINER